Docket No.: 0113476.00122US1
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Steve PAKOLA et al.	Confirmation No.:	3082
Application No.:	10/729,475	Art Unit:	1651
Filed:	December 5, 2003	Examiner:	T. Kim
Title:	PHARMACOLOGICAL VITREOLYSIS		

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. §1.132 OF STEVE PAKOLA, M.D.

Dear Sir:

In connection with the above-referenced patent application, I, Steve Pakola, declare as follows:

1. I received my M.D. degree from University of Pennsylvania School of Medicine in Philadelphia, Pennsylvania. Prior to my current employment with ThromboGenics, I was Associate Director, Cardiovascular Clinical Research, at Boehringer-Ingelheim Pharmaceuticals. Prior to Boehringer-Ingelheim, I also served in senior-level clinical development positions at Quintiles Cardiovascular Therapeutics and Organon, Inc. I am a licensed physician with extensive clinical trial experience, including over 11 years in pharma/biotech clinical development. My curriculum vitae is provided as Attachment 1
2. Currently, I am Chief Medical Officer at ThromboGenics.
3. I am a co-inventor of the above-referenced patent application. I have read and am familiar with the above-referenced patent application. I am also familiar with the Official Action which issued in the above-referenced application on March 26, 2009.

4. In that Official Action, claims 57-61, 64-69, 71, 72 and 80-87 were rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Trese et al (USP 5,304,118) in view of Collen et al (WO 2002/50290) in further view of Wu et al (USP 4,774,087) or Tanaka et al. 2000. I do not agree with this rejection.
5. The rejection of the claims over these references has been maintained and repeated from the prior Official Action. The Official Action asserts that microplasmin allegedly is an art-recognized equivalent of, or a suitable alternative to, plasmin. More specifically, the question raised, and the basis for the rejection, is "whether microplasmin has the same activity (i.e. serine protease activity)for the same purpose (i.e. vitreolysis) as plasmin does." (Official Action at 3). The Official Action alleges that "the" same activity "is drawn to functional and structural characteristics *shared* by plasmin and microplasmin," and that "plasmin and microplasmin both possess fibrinolytic activity (same activity) and they have the identical domain/sequence for such activity, and because of such similarity they can be used for the same purpose of vitreolysis (lysis of collagen)" (emphasis added). (*Id.*) On this basis, the Official Action concludes that "microplasmin and plasmin are considered to be art-accepted equivalent", or that "microplasmin is a suitable alternative to plasmin." (*Id.* at 3-4.)
6. I respectfully disagree with these assertions. First of all, these assertions narrow the proposed concept of "same activity" (entailing functional and structural characteristics) to the function and structure *shared* by microplasmin and plasmin, i.e. the catalytic domain. This overlooks and ignores the structural differences that exist between microplasmin and plasmin, *i.e.,* the absence of the 5 kringle domains comprised in the regulatory chain of plasmin and related changes in actual activity of the catalytic domain due to associated alteration in 3-dimensional structure of the molecule. The absence of these kringle regulatory domains has a profound effect on the function of the catalytic chain. Reducing alleged equivalency between microplasmin and plasmin to an argument based on what both molecules share is only half the story. One skilled in the art would also focus on the functional consequences of structural features that both molecules *don't share* (structural difference). To do otherwise, is an oversimplified and much too narrow view on

microplasmin versus plasmin. Such a focus would be employing hindsight in alleging that the use of microplasmin would have been obvious in view of prior work with plasmin. This approach would be in error. Therefore, and for the reasons outlined hereafter, applicant strongly disagrees with the alleged equivalency between microplasmin and plasmin, as well as with the fact that microplasmin would *a priori* be a suitable alternative to plasmin. Either view can only be arrived at using improper hindsight analysis.

7. In the vitreous of a normal eye, collagen fibers run from the anterior to the posterior pole of the eye, as well as parallel with the internal limiting membrane/lamina (ILM or ILL). Together with other macromolecules such as glucosoaminoglycans (e.g. chondroitin, hyaluronan) and other proteins (e.g. fibrillin-containing microfibrils, fibronectin, opticin), collagen fibers are believed to provide the gelatinous strength to the vitreous. Although collagen fibers naturally tend to stick together they are kept apart from each other in the vitreous. Liquefaction of the vitreous disrupts the network between collagen fibers and other vitreous components and causes collapse of (parts of) the vitreous. The vitreous components are linked to the ILM (an extracellular matrix lining the inner retina). The ILM contains proteoglycans, fibronectin, laminin and different types of collagen (e.g. types I and IV). Laminin and fibronectin are considered to be major ILM-components responsible for gluing the vitreous to the ILM. During the process of posterior vitreous detachment (PVD) the linkage between vitreous and ILM is gradually lost, and eventually the vitreous becomes completely detached from the posterior part of the macula/retina. More information about these aspects of the eye can be found in, e.g., Bishop 2000 (Prog Retin Eye Res 19, 323), Sebag & Balazs 1989 (Invest Ophthalmol Vis Sci 30, 1867) and Sebag 2005 (Trans Am Ophthalmol Soc 103, 473).
8. As a skilled person will appreciate, the efficacy and safety of a protease for inducing vitreolysis (vitreous liquefaction and/or posterior vitreous detachment) depends on a multitude of (not necessarily mutually exclusive) factors. Some of these are discussed in detail herein.

i) Accessibility of protease substrates

9. Factors determining the accessibility to its substrates by a protease include the size of the protease (influencing e.g. diffusion and steric hindrance) and binding/immobilization of the protease by proteins (not necessarily substrate proteins) through, e.g., non-catalytic sites on the protease (influencing sequestration of the protease as well as its diffusion).
10. When applying this factor to microplasmin and plasmin, the patent application describes in paragraph [0081] that microplasmin is a molecule of 26.5 to 29 kDa consisting essentially of the catalytic domain plus a number of amino acid residues from the C-terminus of the regulatory chain but excluding all 5 kringle domains of the regulatory chain as present in plasmin, a molecule of 65 to 83 kDa.
11. Moser et al. 1993 (J Biol Chem 268, 18917) and Stack et al. 1992 (Biochem J 284, 103) describe the binding properties of parts of the plasminogen molecule with proteins (or fragments thereof) which occur in the eye: fibronectin, laminin and collagen IV. The plasmin fragments studied were (i) a fragment spanning kringles 1, 2 and 3 (hereafter "K1-3"), a fragment spanning kringle 4 (hereafter "K4"), and miniplasminogen (kringle 5 + catalytic domain); kringle domains are known to contain lysine-binding sites. Whereas K1-3 and K4 bind fibronectin and collagen IV, miniplasminogen (and, hence, also microplasminogen which lacks kringle 5) does not bind to these proteins. All of K1-3, K4 and miniplasminogen bind to laminin, but binding of miniplasminogen is mainly effectuated through kringle 5; the binding of the catalytic domain (such as in microplasmin) is lysine-independent.
12. The difference in structure between microplasmin and plasmin thus affects the biological properties of the molecules. There is a significant difference between microplasmin and plasmin with regard to their binding to at least the eye proteins fibronectin, laminin and collagen IV. Whereas plasmin binds to all (thus bringing the catalytic domain in close contact with the substrate), microplasmin is potentially binding only to laminin. This difference is due to the difference in structure and lack of kringle domains in microplasmin.
13. The significantly decreased sequestration of microplasmin (vs plasmin) by eye proteins is reasonably expected to increase its diffusion throughout the vitreous and ILM. On the other hand, microplasmin is about 1/3 of the size of plasmin which is also reasonably expected to

contribute to increased diffusion of microplasmin (vs plasmin). Both aspects raise concerns about the to be expected efficacy of microplasmin in vitreolysis as more and/or other targets (relative to plasmin) may be available for proteolytic degradation. Safety concerns also raise as the chances for microplasmin to reach the retinal cells is significantly increased due to its smaller size, and it should be kept in mind that plasmin, when having access to membranes, is disrupting these membranes and is causing cell lysis (Okajima et al. 1995 – J Lab Clin Med 126, 377).

14. Hence, the structural differences between microplasmin and plasmin are reasonably expected to significantly change the efficacy with which eye substrates are proteolytically degraded and warrants very careful monitoring of microplasmin's safety when applied for vitreolysis. The structural and enzymatic properties, and the effect of the structural properties on the enzymatic properties of plasmin apparently make it a safe and efficient protease for pharmacologic vitreolysis. Safety and efficacy of microplasmin for pharmacologic vitreolysis can, however, not *a priori* be established in view of the perturbed balance (relative to plasmin) between catalytic activity *per se* and the influence of the structural changes (lack of kringle regulatory chain) on the catalytic activity, in particular on the *in situ* catalytic activity.
15. At ThromboGenics, we have done work comparing the action of microplasmin and plasmin on *ex-vivo* human vitreous. Vitreous was removed from human eyes and digested with microplasmin and plasmin. After digestion samples were separated on an SDS-PAGE gel followed by Western blot analysis of fibrinogen (Figure 1 in Appendix A) and MMP-9 (Figure 2 in Appendix A).
16. Figure 1 confirms the earlier reported *in vitro* results in that plasmin more efficiently degrades fibrinogen than does microplasmin. The differences for MMP-9 (Figure 2) are even more striking. The control sample (no plasmin or microplasmin added to the *ex-vivo* human vitreous) shows bands of over 260 kDa (presumed multimer of (pro)-MMP9), of around 210 kDa (pro-MMP9 homodimer size reported to be ca. 215 kDa) and of around 52 kDa (size of inactive MMP-9 reported to be ca. 50-60 kDa). The sample digested with microplasmin shows a very prominent new band of about 82 kDa, which corresponds to the reported size

of activated MMP-9 (ca. 82 kDa). This band seems completely absent in the sample digested with plasmin where new bands occur with a size of ca. 65 kDa (which could correspond to activated MMP-9 from which the C-terminal hemopexin-like domain is removed). Overall, the differences detected with an anti-MMP-9 antibody between microplasmin- and plasmin-digested *ex-vivo* human vitreous are striking and very significant. The reports on MMP-9 are by Goldberg et al. 1992 (J Biol Chem 267, 4583) and by Shapiro et al. 1995 (J Biol Chem 270, 6351).

17. The above observations on *ex-vivo* human vitreous clearly and unequivocally show the non-equivalency of microplasmin and plasmin. In addition, knowing that the ubiquitous vitreous/ILM-component collagen IV is a degraded by MMP-9, and given the as yet unknown downstream effect of microplasmin on MMP-9 compared to plasmin, it cannot *a priori* be established that microplasmin is a suitable alternative to plasmin for the purpose of vitreolysis. Microplasmin would not be assumed an equivalent of plasmin given the unpredictability of the effect of microplasmin in vivo in light of the significant differences in enzymatic activity and binding characteristics between the two molecules. These differences are evident from in vitro assessment.
18. The unpredictability in the in vivo setting is dramatically magnified. In the actual setting of pharmacologic vitreolysis, there are various substrates that are important in determining the effect of a pharmacologic vitreolytic agent. These substrates also have different relative importance in the vitreous or the vitreoretinal interface, further complicating the ability to translate any differences in enzymatic activity and binding characteristics observed in testing to actual effect in the relevant clinical setting of pharmacologic vitreolysis in terms of either safety or efficacy. The impact of dramatically different size and binding characteristics on downstream effects of microplasmin and plasmin in vivo could not be predicted. In addition, the differential diffusion and localization of the drug due to dramatically different size and binding characteristics make its effect in the complex biologic system of the vitreous and vitreoretinal interface unpredictable.
19. Those limited aspects that can be hypothesized actually would teach *against* use of microplasmin relative to plasmin, due to safety concerns over greater diffusion through the

ILM and into the retina, due to 1) the greater digestion of the collagen type IV in the ILM, 2) the smaller size of the molecule, and 3) the lack of binding sites including to a2-antiplasmin to retain and/or metabolize the molecule in the vitreous.

(ii) The spectrum of protease activity

20. A number of serine proteases and other broad-spectrum proteases have been evaluated for their suitability in inducing vitreolysis. Serine proteases include trypsin, chymotrypsin and subtilisin. Other broad-spectrum proteases include dispase. These proteases are not without serious adverse effects. Trypsin was reported to cause complete detachment of the ILM from the retina (Hara 1994 – Jpn J Ophthalmol 38, 375), something not wanted in the clinic. Chymotrypsin was reported to destroy the posterior capsule of the lens and to lead to cataract development (Laryukhina & Ziangirova 1977 – Vestn Oftalmol (6), 77) and subtilisin (nattokinase, Takano et al. 2006 – Invest Ophthalmol Vis Sci 47, 2075), being a bacterial protein, has the potential drawback of inducing unwanted immune reactions. Dispase, slightly larger than microplasmin (35.9 to 41 kDa compared to 26.5-29 kDa for microplasmin), has a protease spectrum at least partially overlapping with that of microplasmin: fibronectin and collagen IV are good substrates for dispase. Nevertheless, dispase was reported to exert toxic effects on the retina (Jorge et al. 2003 – Curr Eye Res 26, 107). It is moreover a bacterial protein, thus potentially inducing unwanted immune reactions. In view of the safety issues observed with proteases structurally different from, but sharing the catalytic activity of plasmin, the Examiner's allegation as if microplasmin were an equivalent of or a suitable alternative to plasmin based on the shared serine protease activity is an oversimplified view on the reality of vitreolysis in the eye.
21. Plasmin and microplasmin, like many, many proteases, can lyse collagen to some degree. However, the vitreous (the key site of interest for liquefaction) is composed of numerous other substrates that can be modulated differently. Further, the vitreoretinal interface (the key site of interest for induction of posterior vitreous detachment) has different concentrations of various substrates than are present in the vitreous. Further, there are

certain substrates of proteases, including MMPs such as collagenase, that once activated have their own downstream effect on vitreous and vitreoretinal interface.

22. It is further noted that the broad-spectrum protease activity of microplasmin is reported even after the publication of the instant invention to be of concern relative to safety and efficacy, and a combination of several more specific enzymes is suggested:

> "Indeed, broad acting agents, such as microplasmin, may have a higher likelihood of inducing both a breakdown in vitreous macromolecules and vitreoretinal separation. However, there may also be untoward side effects if the agent's action is too broad. ... Thus, rather than employing a single broad-acting substance such as microplasmin or dispase, a combination of highly specific agents, such as collagenase, hyaluronidase, or chondroitase, may be safer and more effective." (Sebag 2005 – Trans Am Ophthalmol Soc 103, 473; see page 489, last 9 lines of 5th full paragraph).

(iii) The kinetics of protease activity.

23. Clearly, the kinetics of protease activity influences the efficiency of substrate degradation. This process, however, competes with the kinetics of autodegradation of the protease (especially in case of broad-spectrum proteases), as well as with the kinetics of inhibition of the protease by endogenous protease inhibitors. As outlined previously, subtle but complex differences exist between microplasmin and plasmin in *in vitro* degradation of protein substrates present in the eye. As described under (i), this was confirmed by work extending to *ex-vivo* human vitreous (fibrinogen and MMP-9). To what extent these findings can be extrapolated to the more complex true *in situ* situation is unpredictable and *a priori* raises concerns about the safety and efficacy of microplasmin as compared to plasmin for the purpose of vitreolysis.
24. Applicant has also previously reported (see Declaration of Dr. Steve Pakola Under 37 C.F.R. §1.132, submitted February 11, 2008) that microplasmin is inhibited much slower by antiplasmin than plasmin.
25. Applicant has now analyzed *ex-vivo* human vitreous for the presence of antiplasmin by means of Western blotting and this inhibitor is clearly present in the human vitreous (see Figure 3 in Appendix A).

26. In conclusion, the previously and currently presented *in vitro* and *ex-vivo* data demonstrate a plethora of differences in action between microplasmin and plasmin. These differences make it *a priori* unpredictable whether the action of microplasmin for the purpose of vitreolysis would be as safe and efficient as plasmin's action. In preparing for clinical trials with microplasmin for vitreolysis, the applicant performed preclinical studies on human post-mortem eyes (in addition to the preclinical studies on feline eyes and on pig post-mortem eyes already described in the instant patent application) and demonstrated a dose-dependent microplasmin-induced pharmacologic vitreolysis without damaging the underlying retinal tissue/cells, as is illustrated in Figure 4 (in Appendix A).
27. In view of these encouraging results, microplasmin had proceeded into clinical trials, the largest of these trials to date involving 125 patients (1 eye/patient) without PVD but eligible for surgical vitrectomy (29 receiving 25 μg microplasmin/eye; 33 receiving 75 μg microplasmin/eye; 32 receiving 125 μg microplasmin/eye; and 31 receiving an equal volume of saline placebo/eye). A dose-dependent improvement, with best response at the highest microplasmin dose, was observed for a number of clinically relevant endpoints, one of them being the resolution of macular holes without the need of additional surgical vitrectomy. Where none of the eyes of the 15 patients with macular holes and receiving placebo displayed closure of macular holes, such closure was observed in the eyes of 7 out of the 20 patients with macular holes and receiving 125 μg/eye. Closure of macular holes without surgical vitrectomy was also observed in eyes of patients receiving 25μg or 75μg microplasmin/eye, albeit at lower frequency. An illustration of microplasmin-induced closure of a macular hole is given in Figure 5 (in Appendix A). This finding is considered a potentially groundbreaking advance for treatment of these patients, given that there is no available treatment for these patients other than major, invasive eye surgery (vitrectomy) and the risks associated therewith.
28. These results compare as follows with literature data concerning the influence of plasmin on closure of macular holes. Only a single publication was found in which eyes with macular holes were initially treated with plasmin, or a combination of plasmin with gas, i.e. without surgical vitrectomy. Sakuma et al. 2005 (Eur J Ophthalmol 15, 787) reported that none of

the 8 eyes treated with plasmin, or with plasmin combined with gas, led to closure of macular holes (see, e.g., "Conclusions" in Abstract), notwithstanding successful vitreous liquefaction and PVD. Closure of macular holes was achieved only after performing additional conventional surgical vitrectomy (see, e.g., Figure 2 in Sakuma et al. 2005). A reported benefit of plasmin in this procedure was the simplification and shortening of the surgical procedures. Another publication, by Trese et al. 2000 (Ophthalmology 107, 1607) reported on the closure of idiopathic macular holes. In contrast to the applicant's work and Sakuma et al. 2005, the procedure, however, involved immediate classical surgical vitrectomy and gas injection following incubation with injected plasmin. The reported benefit of plasmin concerned the reduction of operative time (see "Conclusions" in Abstract).

29. Three other publications (Margherio et al. 1998 – Ophthalmology 105, 1617; Chow et al. 1999 – Retina 19, 405; and Wu et al. 2007 – Am J Ophthalmol 144, 668) reported on the closure of traumatic macular holes. In all cases the procedure involved injection and incubation with plasmin immediately followed by conventional surgical vitrectomy and exchange of the vitreous fluid with gas or silicone oil. Moreover, Chow et al. 1999 reported the absence of a statistically significant effect of plasmin in achieving anatomic closure of traumatic macular holes (see page 407, left-hand column, last sentence of 4th full paragraph).
30. The results of Applicant's clinical trials using microplasmin for vitreolysis show that microplasmin is unexpectedly safe and efficient in inducing vitreous liquefaction and PVD. As outlined above, this finding is not trivial and could, given the vast differences between microplasmin and plasmin, not be predicted from earlier similar work performed with plasmin. A further unexpected outcome of the clinical trials revealed that the process of vitreolysis by microplasmin must be different from that of vitreolysis by plasmin. This follows from the stunning finding that the action of microplasmin, but not the action of plasmin, is capable of inducing closure of macular holes in the absence of any surgical vitrectomy.
31. This unexpected beneficial clinical advantage of microplasmin over plasmin clearly supports applicant's arguments about the differences between microplasmin and plasmin, as well as

the argued unpredictability of substituting microplasmin for plasmin with a reasonable expectation of success (in terms of safety and efficacy). Moreover, all previously and currently presented *in vitro*, *ex vivo* and *in situ* data unequivocally illustrate that microplasmin and plasmin are two proteases not only with a different structure but also with different activity (e.g., different enzymatic activity, different binding and inactivation characterization and diffusion rates). Such differences are significant and render the effects of microplasmin unpredictable in the complex biologic system of both the vitreous and the vitreoretinal interface.

32. Many proteases are capable of liquefying the vitreous and/or inducing PVD. And although both plasmin and microplasmin are capable of effectuating both vitreous liquefaction and induction of PVD, the resulting liquefaction and PVD must differ between microplasmin and plasmin as only a difference can explain why microplasmin is efficient in inducing closure of macular holes in the absence of surgical vitrectomy and why plasmin in ineffective in this setting.

33. Hence, my overall conclusions are (i) that microplasmin and plasmin cannot be considered as art-recognized equivalents and (ii) that the current invention unexpectedly established microplasmin to be not only a suitable but moreover also an improved alternative to plasmin.

34. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 25-SEP-2008

Steve Pakola, M.D.

ATTACHMENT 1

Curriculum Vitae of Dr. Stephen Pakola

Stephen J. Pakola, M.D.
11 Meadow Way
Irvington, NY 10533
Home Phone: 1-914-674-1947
email: steve.pakola@thrombogenics.com

Professional Summary: A licensed physician with over 13 years of clinical trial experience, including over 11 years of full-time Pharma/Biotech experience in roles of increasing responsibility (currently Chief Medical Officer of ThromboGenics and member of the Board of Directors of ThromboGenics Ltd.).

Education

M.D., University of Pennsylvania, Philadelphia, PA, 1994
*Alpha Omega Alpha (national medical honor society) elected 1993
*Charles A. Oliver Prize (awarded annually to a graduating medical student at the University of Pennsylvania), 1994

B.A., University of Pennsylvania, Philadelphia, PA, 1990
*Summa cum laude (GPA-3.9) with honors in biology
*Phi Beta Kappa, elected 1989

Professional Experience

5/00-Present	Chief Medical Officer (Promoted from Senior VP in 6/06, promoted from VP on 7/02) Member of the Board of Directors, ThromboGenics Ltd. *Responsible for the company's worldwide clinical development programs (vitreoretinal disease, AMI, ischemic stroke, DVT prophylaxis, oncology, and other programs to enter clinical development in future) *Program Leader for microplasmin-vitreoretinal program.
11/98-5/00	Associate Director, Cardiovascular Clinical Research, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT *U.S. clinical leader for oral direct thrombin inhibitor (BIBR) program *Global Medical Leader of clinical development program for BIBB 1464, a lipid-lowering agent. *Named to Clinical Expert Committee for risk factor intervention (internal expert group to review discovery/development plans for Boehringer Ingelheim worldwide)
6/97-11/98	Associate Director, Organon, Inc. CNS Clinical, West Orange, NJ

*Clinical Development Team Leader for gepirone (prior to departure, in charge of the preparation of the clinical section of NDA and clinical development plan for US)
*Unit head award recipient, 1st quarter, 1998 (awarded by executive director of clinical department to employees who have made significant contributions to the department)
*Study manager for 2 multicenter clinical trials (phase III ten-site trial and a phase IV, five-site trial)
*Responsible for safety related issues for U.S. phase II-IV trials on all CNS projects.

1996-1997 Senior Medical Scientist, Cardiovascular Division, Quintiles, Inc., Research Triangle Park, N.C.

*Project Medical Officer on all clinical trials in the U.S. Cardiovascular division, including phase I through global phase III trials (including GUARDIAN trial)
*Presented at FDA Advisory Committee meeting, as well as numerous investigator meetings

1995-1996 Research Scientist and Ophthalmology Resident, University of Pennsylvania, F.M. Kirby Center for Gene Therapy

*Planned and performed experiments in biotechnology arena, including virus vector gene transfer techniques
*Authored multiple publications (including one book chapter), and presented work at 4 national meetings
*Served as a reviewer for a medical journal

1994-1995 Medical Intern Physician, Presbyterian Medical Center (Affiliate of University of Pennsylvania Medical Center), Philadelphia, PA

Other

*99% on GMAT (Graduate Management Admission Test)
*Unrestricted license to practice medicine (License: NC 9700351)
*Married with 3 children

Original Papers

Pakola SJ and Grunwald JE: Effects of oxygen and carbon dioxide on human retinal circulation. Investigative Ophthalmology & Visual Science 34(10): 2866-70, 1993.

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Balcer L, Galetta S, Bagley L, Pakola S: Localization of traumatic oculomotor nerve palsy to the midbrain exit sight by M.R.I. Am. J. Ophthalmol. 122(3): 437-9, 1996.

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Rasmussen RS, Overgaard K, Pakola S, Boysen G. Microplasmin may improve recovery in a rat embolic stroke model. Neurol Res. 2007 Jun 19; [Epub ahead of print].

Sakuma T, Tanaka M, Mizota A, Junji Inoue J, Pakola S. Safety of in vivo pharmacologic vitreolysis with recombinant microplasmin in rabbit eyes. Invest Ophthalmol Vis Sci. 2005 Sep;46(9):3295-9.

Chapters

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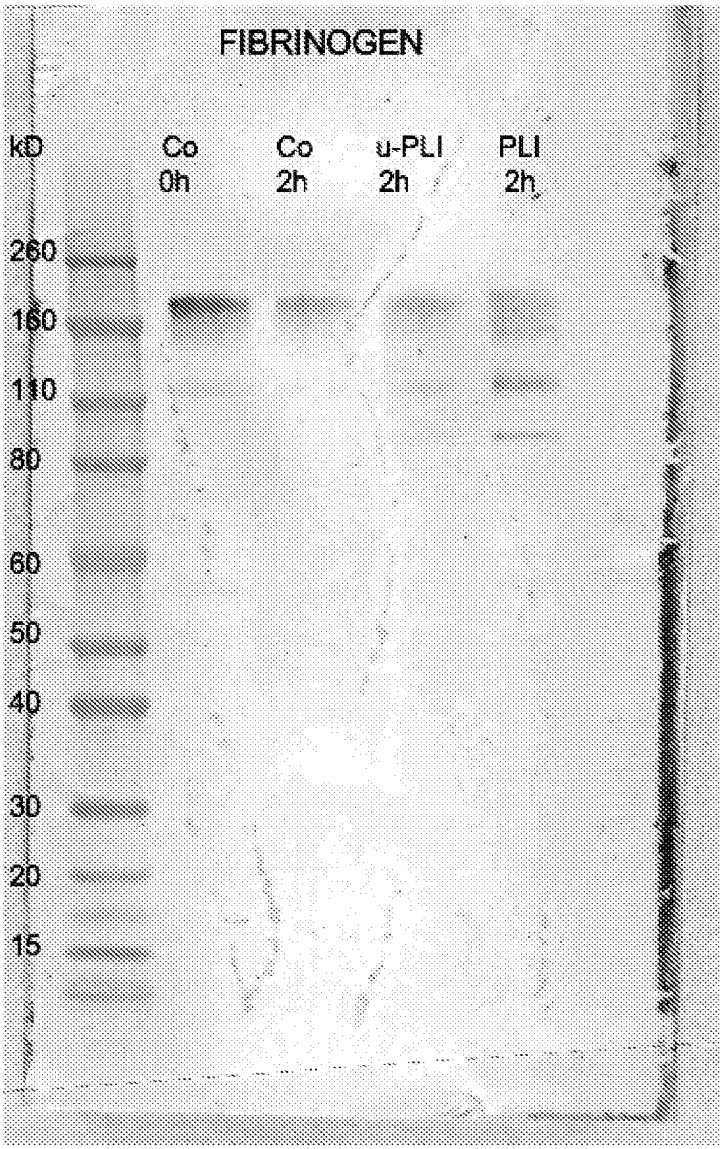
APPENDIX A

Figure 1



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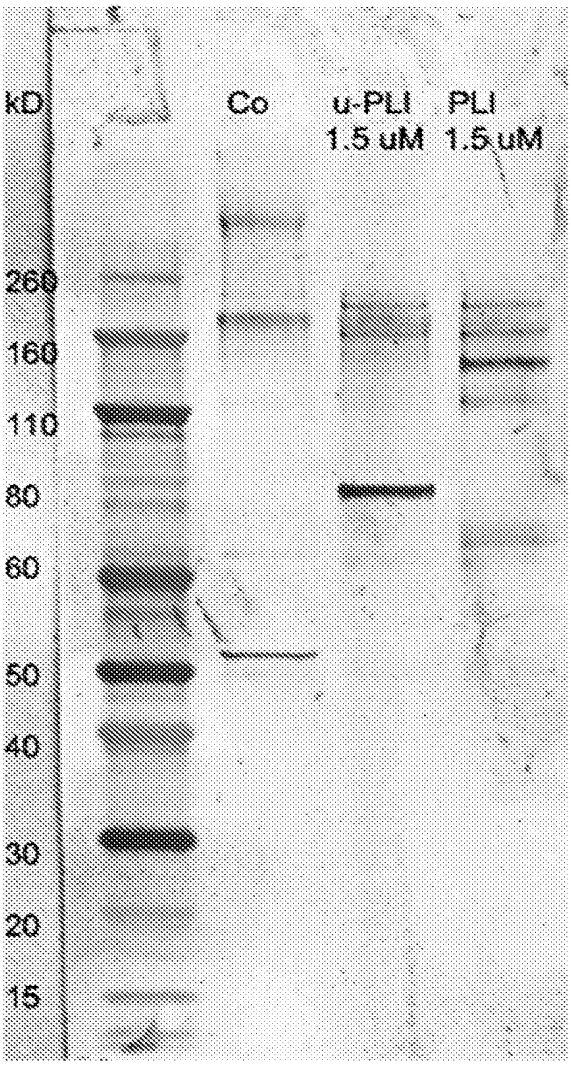
APPENDIX A

Figure 2



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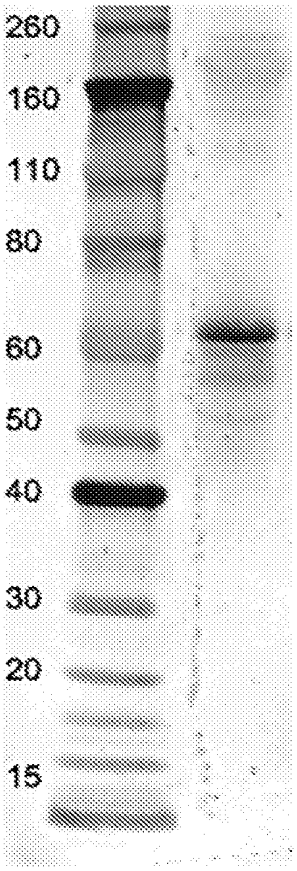
APPENDIX A

FIGURE 3



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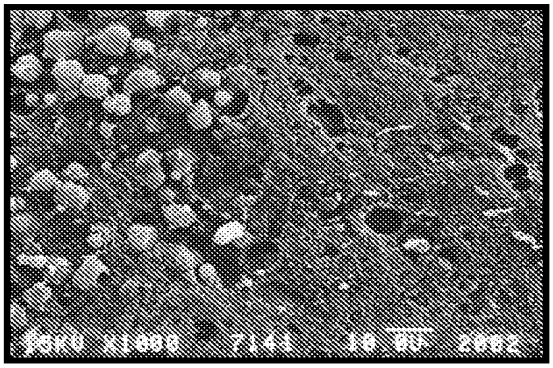
APPENDIX A

FIGURE 4



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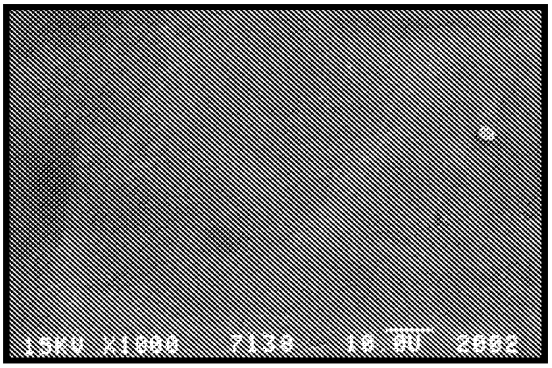

MICROPLASMIN-TREATED

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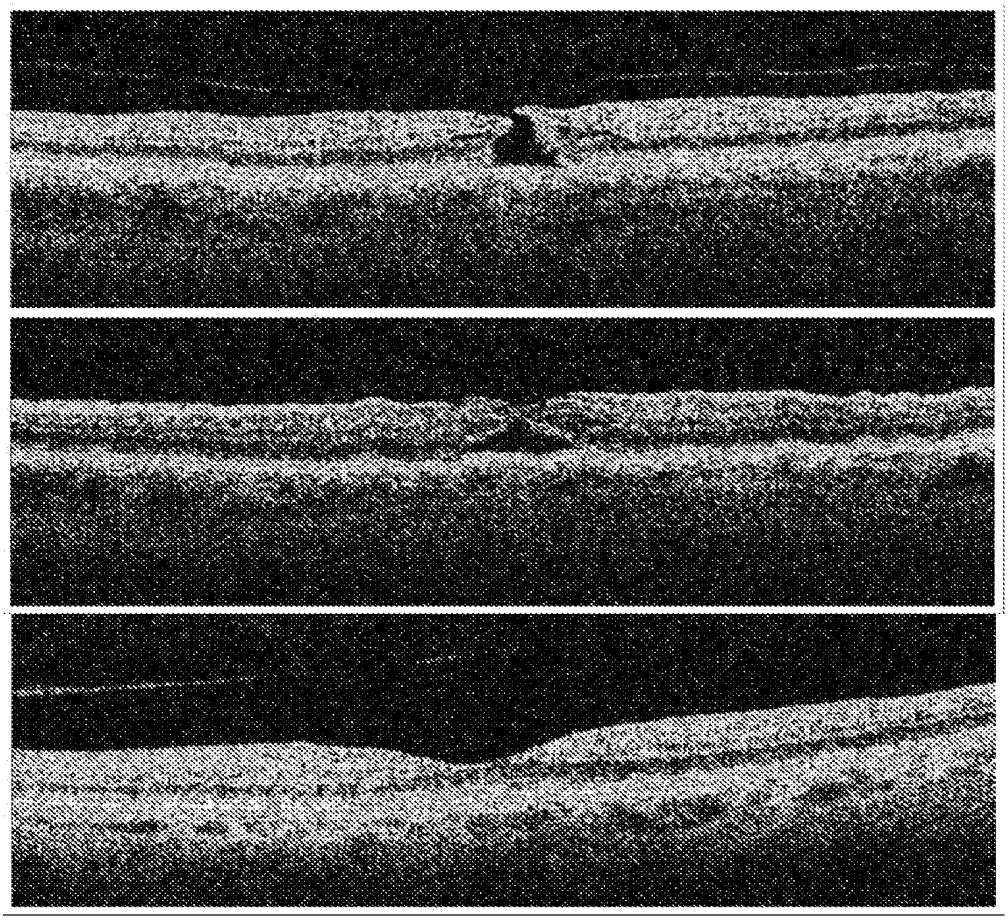
APPENDIX A

FIGURE 5



pre-injection

7days post injection

1 month post injection

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